Hepatoprotective effects of systemic ER activation

Spheroid RNA-seq - Downstream analysis

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```
# source and library import
source('code/00_helper_functions.R')
library(clusterProfiler)
library(tidyverse)
library(org.Hs.eg.db)
library(Mfuzz)
library(biomaRt)
library(GenomicRanges)
library(ComplexHeatmap)
```

```
# color palettes
colPals <- list()
colPals$RdBu <- rev(RColorBrewer::brewer.pal(n=11, name = 'RdBu'))
colPals$BrBG <- rev(RColorBrewer::brewer.pal(n=11, name = 'BrBG'))
colPals$UpDown <- setNames(colPals$RdBu[c(10,2)],
                           c('up', 'down'))
```

Load data

```
# spheroid TPM-normalized expression
spheroid_tpm <- read.table(
  file = 'results/spheroid_TPM_norm_counts.txt',
  sep = '\t',
  header = T,
  quote = ''
)
# spherioid differential expression results
inhibitor_RDS <- readRDS("results/Spheroid_inhibitors_DEGlists.rds")

universe <- inhibitor_RDS$unfilt$TEADap_vs_control %>% pull("external_gene_name")

TEADap_inh_vs_control <- inhibitor_RDS$filt$TEADap_vs_control
TEADsf_inh_vs_control <- inhibitor_RDS$filt$TEADsf_vs_control

# human gene promoter regions (ensembl 109)
promoter_regions <- read.table(
  file = "data/ensembl_hsap_feb2023_promoter_regions.bed",
  sep = "\t",
  quote = "",
  header = F)
colnames(promoter_regions) <- c(
  'chromosome', 'promoter_start', 'promoter_end', 'ensembl_gene_id',
  'external_gene_name', 'ensembl_transcript_id', 'strand', 'gene_biotype', 'description')

# TEAD1 binding sites geneome-wide (HOCOMOCOv11 + PWMScan)
tead1_tfbs <- read.table(
  file = "data/TEAD1_tfbs_hg38_hocomoco_v11_pwmscan.bed",
  sep = "\t",
  quote = "",
  header = F)
colnames(tead1_tfbs) <- c('chromosome', 'start', 'end', 'sequence', 'score', 'strand')

# mouse-human orthologs (ensembl 105)
mouse_human_orthologs <- read.table(
  file = 'data/ensembl_mmus_hsap_dec2021_orthologs.tsv',
  sep = '\t',
  header = TRUE,
  quote = '')
```

```r
# mouse differentially expressed genes
DEGs_mouse <- readRDS('results/bulkRNAseq_mmus_DEGs.rds')
```

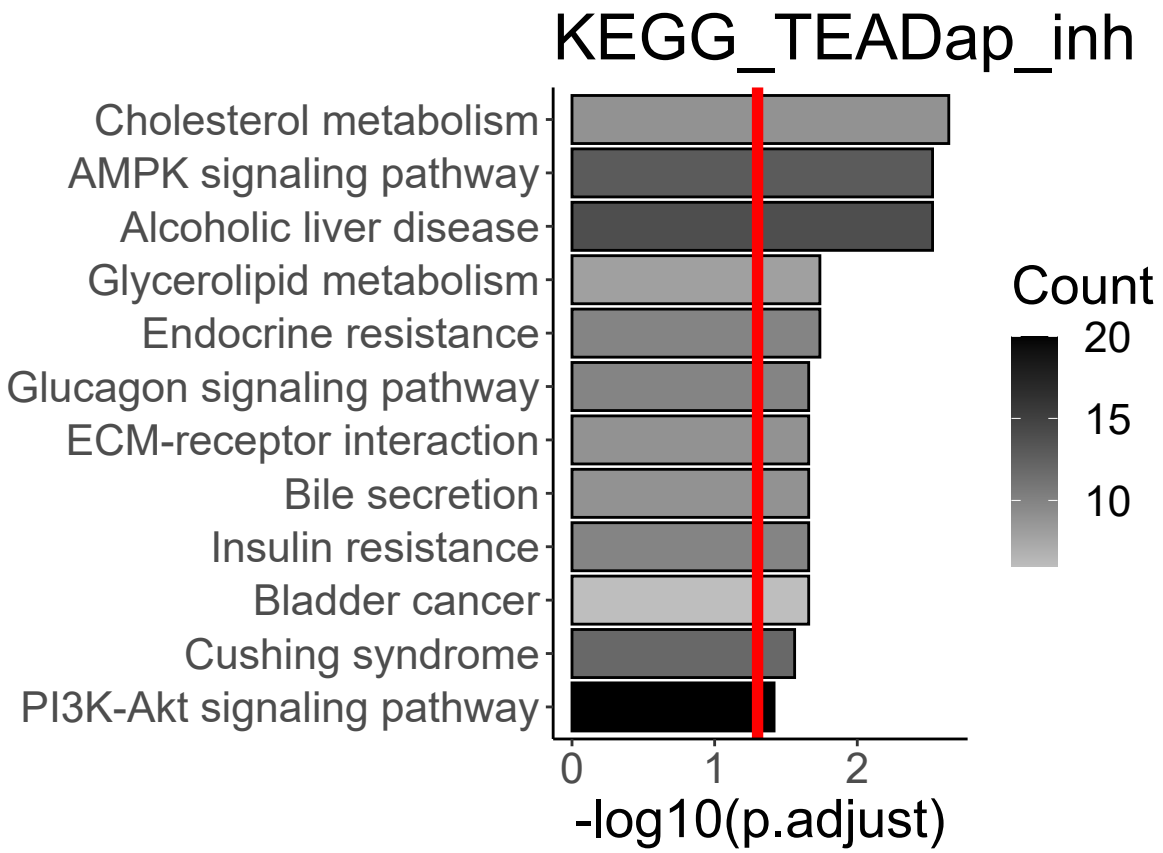
KEGG over-representation analysis

```r
TEADap_inh_vs_control_entrez <- bitr(TEADap_inh_vs_control$ensembl_gene_id, fromType = "ENSEMBL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db")
TEADsf_inh_vs_control_entrez <- bitr(TEADsf_inh_vs_control$ensembl_gene_id, fromType = "ENSEMBL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db")

KEGG_TEADap_inh <- enrichKEGG(gene = TEADap_inh_vs_control_entrez$ENTREZID,
          organism = "hsa",
          keyType = "kegg",
          pAdjustMethod = "BH",
          qvalueCutoff = 0.05)
KEGG_TEADap_inh <- as.data.frame(KEGG_TEADap_inh@result)
#write.table(KEGG_TEADap_inh, "results/KEGG_TEADap_vs_control_FigS8A.txt", sep="\t", quote=F, row.names = F)

KEGG_TEADsf_inh <- enrichKEGG(gene = TEADsf_inh_vs_control_entrez$ENTREZID,
          organism = "hsa",
          keyType = "kegg",
          pAdjustMethod = "BH",
          qvalueCutoff = 0.05)
KEGG_TEADsf_inh <- as.data.frame(KEGG_TEADsf_inh@result)


#This function tells you the gene symbols in each KEGG pathway (changed by TEADap)
gimme_SYMBOL_from_KEGG <- function(table, pathway) {
 table_subset <- table %>%  filter(Description==pathway) %>% pull("geneID")
 split_entrez <- unlist(strsplit(as.character(table_subset), "/"))
 symbols <- bitr(split_entrez, fromType = "ENTREZID", toType = "SYMBOL", OrgDb = "org.Hs.eg.db")
 symbols <- symbols %>%  mutate(Pathway = pathway)
 symbols

}

# This code exports the genes inside the top12 KEGG pathways of TEADap vs control with the corresponding human-readable symbols.
KEGG_top12_pathway_geneList <- list()
for (i in KEGG_TEADap_inh$Description[1:12]) {
  KEGG_top12_pathway_geneList[[i]] <- gimme_SYMBOL_from_KEGG(table=KEGG_TEADap_inh, pathway=i)
}

#saveRDS(KEGG_top12_pathway_geneList, "results/top12KEGGpathways_gene_content.rds")
```

Plotting the KEGG pathways

```r
# Import the KEGG table (run in June 2023, KEGG version from April)
KEGG_TEADap_inh <- read.delim("results/KEGG_TEADap_vs_control_FigS8A.txt")

# Note: This following section is looped, so one can technically plot many results at once.
list_KEGG <- list(KEGG_TEADap_inh=KEGG_TEADap_inh)

combined_genesets <- list()

for (i in names(list_KEGG)) {

combined_genesets[[i]] <- list_KEGG[[i]] %>%
              dplyr::arrange(p.adjust) %>%
              dplyr::slice(1:12)

names(combined_genesets[[i]])
order <- list()
order[[i]] <- combined_genesets[[i]] %>% dplyr::pull(Description)
# Plot and order by p.adjust
ggplot_bar <- ggplot(combined_genesets[[i]], aes(x=factor(Description, levels=order[[i]]), y=-log10(p.adjust), fill=Count)) +
  geom_col(color="black") +
  coord_flip() +
  scale_x_discrete(limits=rev) +
  #scale_y_continuous("-log10") +
  scale_fill_gradient(high = "black", low="grey") +
  theme_classic() +
  theme(text=element_text(size=20)) +
  geom_hline(yintercept = -log10(0.05), color="red", linewidth=2) +
  xlab("") +
  ggtitle(i)
```

```
date <- gsub("-", "", Sys.Date())

print(ggplot_bar)

#ggsave(filename = paste0("results/","SupplFig8A_KEGG_RNAseq_top12_", i, ".pdf"), width=10, height=7)

}
```



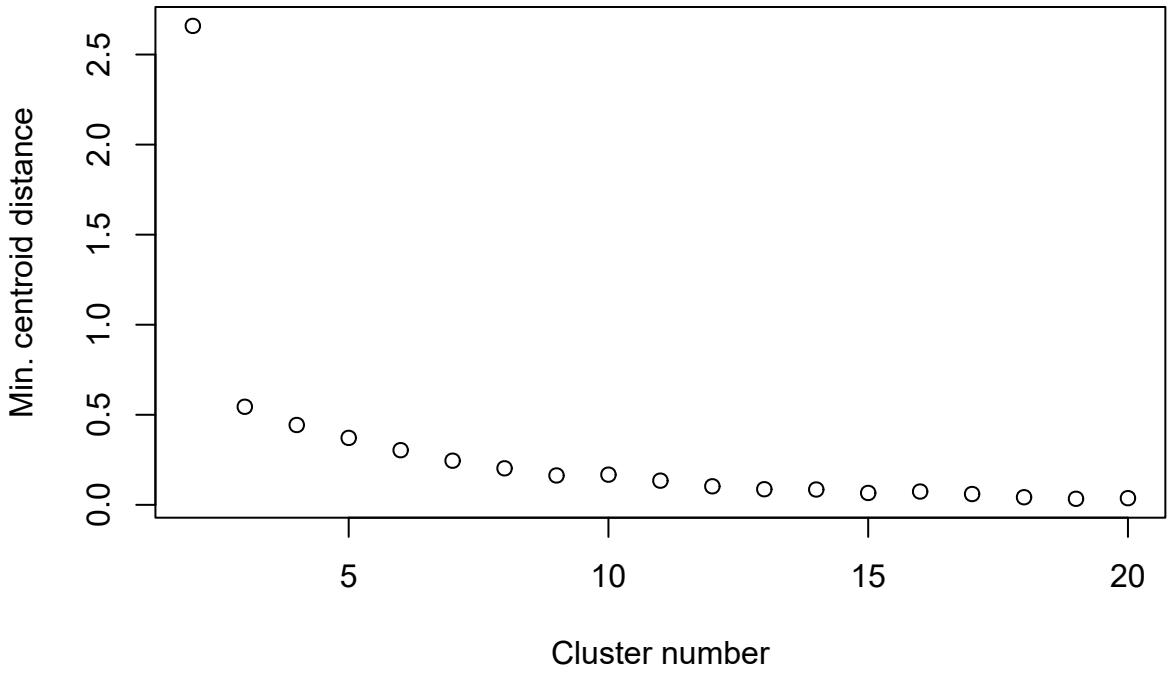
Gene expression trend analysis

```
# CLUSTERING WITH ONLY Inhibitors DEG

# Clustering of expression profiles
DESeq2_DEGs_clust <- list(TEADap_inh_vs_control, TEADsf_inh_vs_control)

names(DESeq2_DEGs_clust) <- c("TEADap_inh_vs_control", "TEADsf_inh_vs_control")

DEGs_union <- lapply(DESeq2_DEGs_clust, function(x) x$ensembl_gene_id) %>%
  unlist() %>%
  unique()

# TPM - normalized table with the average TPM (computed in Spheroid DESeq2 analysis script)
nonNorm_Counts <- read.delim("results/spheroid_TPM_norm_counts_mean.txt")

TPM_Normalized <- nonNorm_Counts %>% tibble::column_to_rownames("ensembl_gene_id") %>%
  dplyr::select(-description, -external_gene_name)
colSums(TPM_Normalized)
```

```
##  control   TEADap   TEADsf
## 999994.4 999992.2 999990.9
```

```
eset <- TPM_Normalized %>%
  dplyr::filter(row.names(.) %in% DEGs_union)

# zscore data (mean=0, sd=1)
eset <- new('ExpressionSet', exprs = as.matrix(eset)) %>%
```

```
  Mfuzz::standardise()

# estimate fuzzifier parameter for clustering
m_eset <- Mfuzz::mestimate(eset)

# determine cluster number with minimum centroid distance
Mfuzz::Dmin(eset, m = m_eset, crange = seq(2,20,1), repeats = 5)
```



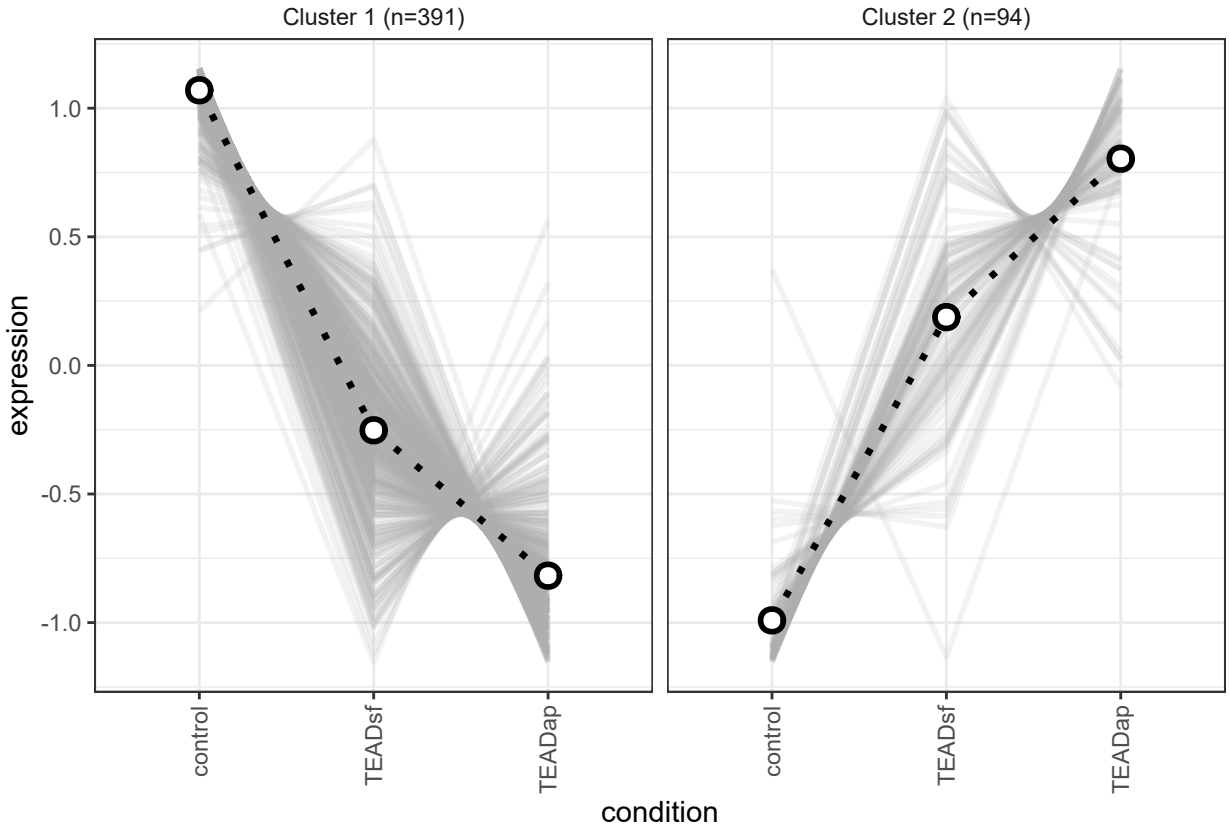

```
##  [1] 2.65902986 0.54449207 0.44331159 0.37172963 0.30349839 0.24511565
##  [7] 0.20275027 0.16310444 0.16764470 0.13431390 0.10297170 0.08671129
## [13] 0.08513811 0.06602575 0.07389691 0.06003509 0.04228936 0.03360471
## [19] 0.03708658
set.seed(3452)

# generate mfuzz clusters (n=4)
clusters <- mfuzz(eset, c = 2, m = m_eset)

# check correlation of cluster centroids
cor(t(clusters[[1]]))
```

```
##            1          2
## 1  1.0000000 -0.9988336
## 2 -0.9988336  1.0000000
# get cluster membership values of genes
cluster_memberships <- acore(eset, cl = clusters, min.acore = 0.0)

# assign to cluster with top membership value
cluster_memberships <- do.call(rbind,
                               lapply(seq_along(cluster_memberships),
                                      function(x) {data.frame(CLUSTER=x,
                                                              cluster_memberships[[x]])})) %>%
  dplyr::mutate(CLUSTER=dplyr::recode(CLUSTER, !!!setNames(c(2,1), seq(1,2,1))))

# check number of genes per cluster
cluster_gene_counts <- table(cluster_memberships$CLUSTER)
cluster_gene_counts
```

```
##
```

```
##   1   2
## 391  94
```

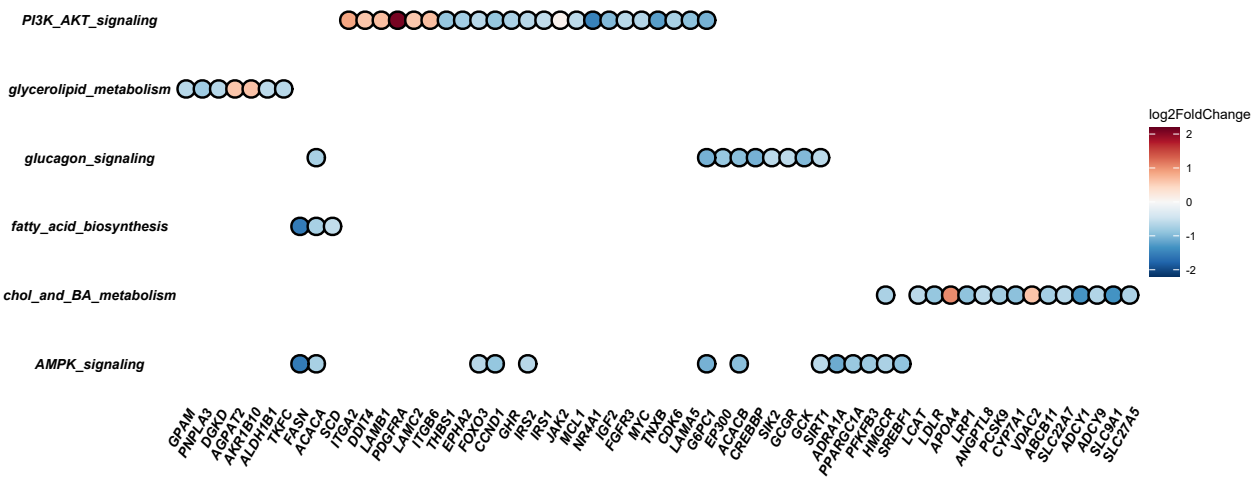
```
DEG_clusters <- list()
# extract gene profiles and cluster assignments
DEG_clusters$genes <- as.data.frame(exprs(eset)) %>%
  tibble::rownames_to_column(var = 'geneID') %>%
  tibble::add_column(GeneSymbol = .$geneID, .after = 'geneID') %>%
  dplyr::mutate(GeneSymbol=dplyr::recode(GeneSymbol,
                                         !!!setNames(inhibitor_RDS$unfilt$TEADap_vs_control$external_gene_name,
                                                     inhibitor_RDS$unfilt$TEADap_vs_control$ensembl_gene_id))) %>%
  merge(cluster_memberships, by.x = 'geneID', by.y = 'NAME', sort = F)

# extract cluster centroid profiles
DEG_clusters$centroids <- as.data.frame(clusters$centers) %>%
  tibble::add_column(geneID = paste0('centroid_', c(2,1)), .before = 'control') %>%
  tibble::add_column(GeneSymbol = paste0('centroid_', c(2,1)), .before = 'control') %>%
  dplyr::mutate(CLUSTER=c(2,1),
                MEM.SHIP=1) %>%
  arrange(CLUSTER)

df <- DEG_clusters$genes %>%
  dplyr::bind_rows(DEG_clusters$centroids) %>%
  tidyr::pivot_longer(cols = c("control", "TEADsf", "TEADap"),
                      names_to = 'condition',
                      values_to = 'expression') %>%
  dplyr::mutate(CLUSTER=factor(CLUSTER, levels = 1:2),
                condition=factor(condition, levels = c("control", "TEADsf", "TEADap")))

cluster_names <- c('1' = paste0("Cluster 1 (n=",cluster_gene_counts[1],")"),
                   '2' = paste0("Cluster 2 (n=",cluster_gene_counts[2],")"))

ggplot(df, aes(x=condition, y=expression, color=CLUSTER, group=geneID)) +
  geom_line(data = subset(df, !grepl('centroid', GeneSymbol)), size = 1,  color=alpha('#AEAEAE', 0.15)) +
  geom_line(data = subset(df, grepl('centroid', GeneSymbol)), size = 1.2, linetype="dotted", color="black") +
  geom_point(data = subset(df, grepl('centroid', GeneSymbol)), shape=21, size=3, stroke=1.5, fill='white', color="black") +
  #scale_color_manual(values = colPals$clusters) +
  facet_wrap(~CLUSTER, nrow = 1, labeller = as_labeller(cluster_names)) +
  theme_bw() +
  theme(strip.background = element_blank(),
        axis.text.x=element_text(angle=90, hjust=0.95, vjust=0.5))
```




```
#ggsave("cMeans_clustering_inhibitors_2clusters.pdf", width = 23, height=10, units="cm")
#write.table(DEG_clusters$genes, "cMeans_clustering_inhibitors_2clusters.txt", sep="\t", row.names = F, quote = F)
```

Gene expression fold change in key pathways (TEADap vs control)

```
pathway_genes <- list(
  glycerolipid_metabolism = c('GPAM','PNPLA3','DGKD','AGPAT2','AKR1B10','ALDH1B1','TKFC'),
  fatty_acid_biosynthesis = c('FASN','ACACA','SCD'),
  PI3K_AKT_signaling = c('ITGA2','DDIT4','LAMB1','PDGFRA','LAMC2','ITGB6','THBS1',
                         'EPHA2','FOXO3','CCND1','GHR','IRS2','IRS1','JAK2','MCL1',
                         'NR4A1','IGF2','FGFR3','MYC','TNXB','CDK6','LAMA5','G6PC1'),
  glucagon_signaling = c('EP300','ACACA','ACACB','CREBBP','SIK2','GCGR','G6PC1','GCK','SIRT1'),
  AMPK_signaling = c('FOXO3','ADRA1A','ACACA','PPARGC1A','FASN','ACACB','G6PC1',
                     'PFKFB3','HMGCR','IRS2','SIRT1','CCND1','SREBF1'),
  chol_and_BA_metabolism = c('HMGCR','LCAT','LDLR','APOA4','LRP1','ANGPTL8','PCSK9','CYP7A1',
                                          'VDAC2','ABCB11','SLC22A7','ADCY1','ADCY9','SLC9A1','SLC27A5')
)

df <- lapply(names(pathway_genes), function(x){
  inhibitor_RDS$unfilt$TEADap_vs_control %>%
    filter(external_gene_name %in% pathway_genes[[x]]) %>%
    mutate(pathway = x,
           external_gene_name = factor(external_gene_name, levels=pathway_genes[[x]])) %>%
    dplyr::select(pathway, external_gene_name, log2FoldChange) %>%
    arrange(external_gene_name)
}) %>% bind_rows()

ggplot(df, aes(x=external_gene_name, y=pathway, fill=log2FoldChange)) +
  geom_point(shape=21, size=4, stroke=1) +
  scale_fill_gradientn(colours = colPals$RdBu, limits=c(-2.2,2.2)) +
  theme_void(base_size = 8) +
  theme(axis.text = element_text(face = 'bold.italic'),
        axis.text.x=element_text(angle=60, hjust=0.9, vjust=0.9))
```



Promoter binding sites

```
# promoter regions
promoters_gr <- with(promoter_regions, GRanges(chromosome, IRanges(promoter_start, promoter_end), strand = strand))

# binding site regions
tead1_tfbs_gr <- with(tead1_tfbs, GRanges(chromosome, IRanges(start, end), strand = strand))

# overlap analysis ignoring strand information
overlap <- countOverlaps(promoters_gr, tead1_tfbs_gr, ignore.strand = TRUE)

# add the counts to the promoter data frame (promoter with max. binding sites per gene selected)
promoter_tead1_overlap <- promoter_regions %>%
  mutate(tead1_bs = overlap) %>%
  group_by(ensembl_gene_id) %>%
  slice_max(tead1_bs, with_ties = F)

# separate promoters by TEADap inhibitor DEGs and non-DEGs
df <- promoter_tead1_overlap %>%
  dplyr::select(ensembl_gene_id, external_gene_name, gene_biotype, description, tead1_bs) %>%
  mutate(type = ifelse(ensembl_gene_id %in% TEADap_inh_vs_control$ensembl_gene_id, 'DEGs', 'Non-DEGs'))

# get random background sets for permutation test
# set.seed(23)
non_DEG_set <- df %>% dplyr::filter(type=='Non-DEGs') %>% pull(ensembl_gene_id) %>% unique()
DEG_set <- df %>% dplyr::filter(type=='DEGs') %>% pull(ensembl_gene_id) %>% unique()
# random_genes <- lapply(seq(1,1000), function(x) sample(x = non_DEG_set, size = length(DEG_set)))
# saveRDS(random_genes, file = 'results/spheroid_TEADap_random_promoter_sets.rds')
random_genes <- readRDS(file = 'results/spheroid_TEADap_random_promoter_sets.rds')

# TEAD1 binding sites in DEGs vs non-DEG sets
non_DEG_bs <- lapply(random_genes, function(x){
  df %>%
    dplyr::filter(ensembl_gene_id %in% x) %>%
    pull(tead1_bs) %>%
    mean()
}) %>% unlist() %>% as.data.frame() %>% rename('.' = 'avg_bs')

DEG_bs <- df %>%
    dplyr::filter(ensembl_gene_id %in% DEG_set) %>%
    pull(tead1_bs) %>%
    mean()

print('Average binding sites in non-DEG sets:')
```

```
## [1] "Average binding sites in non-DEG sets:"
```

```
summary(non_DEG_bs)
```

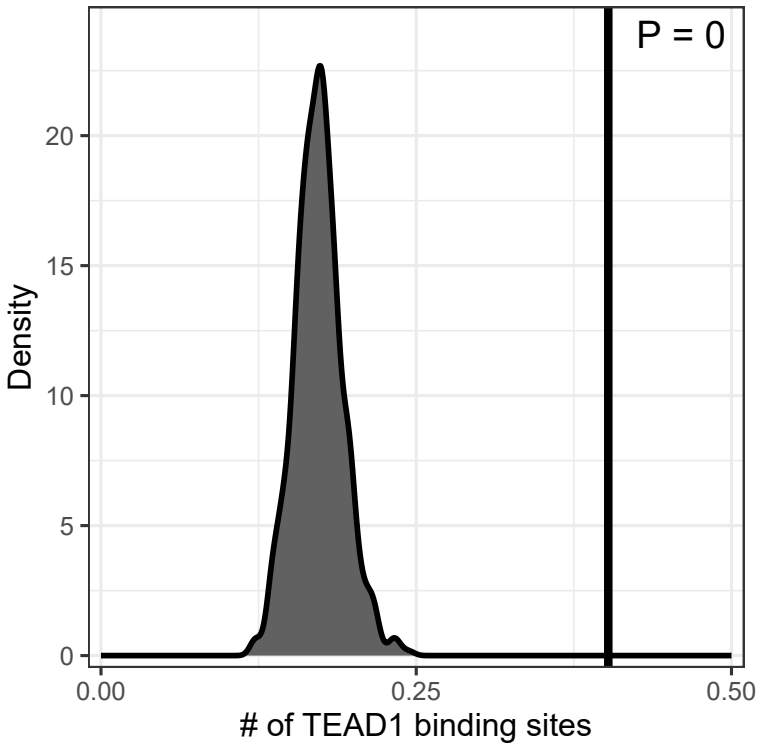
```
##      avg_bs
##  Min.   :0.1195
##  1st Qu.:0.1609
##  Median :0.1724
##  Mean   :0.1727
```

```
##  3rd Qu.:0.1839
##  Max.   :0.2460
```

```
paste('Average binding sites in TEADap inhibitor DEG set:', DEG_bs)
```

```
## [1] "Average binding sites in TEADap inhibitor DEG set: 0.402298850574713"
```

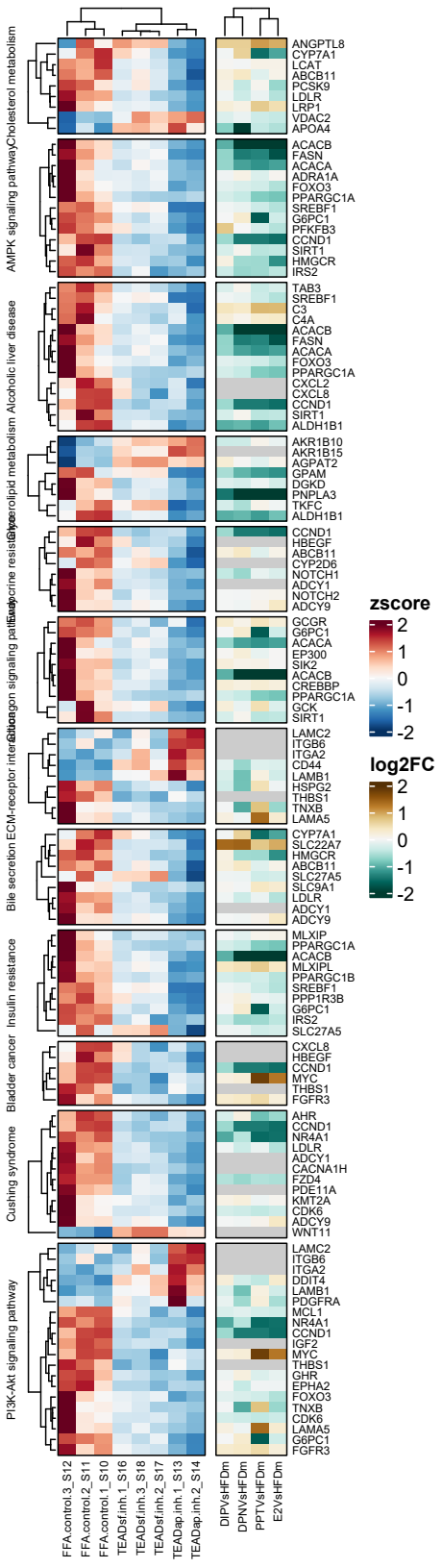
```
pval <- tailFraction(DEG_bs, non_DEG_bs$avg_bs, tail = 'right')

ggplot(non_DEG_bs, aes(x = avg_bs)) +
  geom_density(lwd = 1, colour = 'black',
               fill = '#616161', alpha = 1) +
  geom_vline(xintercept = DEG_bs, lwd = 1.5) +
  scale_x_continuous(limits = c(0,0.5),
                     breaks = c(0,0.25,0.5),
                     expand = expansion(mult = c(.02, .02))) +
  scale_y_continuous(expand = expansion(mult = c(.02, .1))) +
  annotate("text",  x=Inf, y = Inf, label = paste('P =',pval), vjust=1.5, hjust=1.2, size=5) +
  xlab('# of TEAD1 binding sites') +
  ylab('Density') +
  theme_bw(base_size = 12)
```



Expression heatmap top 12 KEGG pathways

```
top12_kegg <- readRDS('results/top12KEGGpathways_gene_content.rds')

df <- dplyr::bind_rows(top12_kegg) %>%
  dplyr::rename(external_gene_name = SYMBOL) %>%
  dplyr::inner_join(spheroid_tpm, by = 'external_gene_name') %>%
  dplyr::mutate(external_gene_name_unique = uniquify(external_gene_name, sep='_'))

mouse_human_orthologs <- mouse_human_orthologs %>%
  dplyr::filter(!GeneSymbol_human == '') %>%
  dplyr::filter(!GeneSymbol_mouse == '')

selected_orthologs <- df %>%
  dplyr::select(external_gene_name) %>%
  dplyr::filter(!duplicated(external_gene_name)) %>%
  dplyr::mutate(external_gene_name_mouse = dplyr::recode(external_gene_name,
                                                         !!!setNames(mouse_human_orthologs$GeneSymbol_mouse,
                                                                     mouse_human_orthologs$GeneSymbol_human)))
```

```r
mouse_ERtreatmentvsHFDm <- lapply(names(DEGs_mouse$unfilt[5:8]), function(x){
  DEGs_mouse$unfilt[[x]] %>%
    dplyr::select(external_gene_name, log2FoldChange) %>%
    dplyr::rename(!!x := log2FoldChange)
}) %>% dplyr::bind_cols() %>%
  dplyr::select(-paste('external_gene_name', seq(3,7,2), sep='...')) %>%
  dplyr::rename(external_gene_name = 'external_gene_name...1') %>%
  dplyr::filter(external_gene_name %in% selected_orthologs$external_gene_name_mouse) %>%
  dplyr::mutate(external_gene_name =dplyr::recode(external_gene_name,
                                                  !!!setNames(mouse_human_orthologs$GeneSymbol_human,
                                                              mouse_human_orthologs$GeneSymbol_mouse)))

df <- df %>%
  left_join(mouse_ERtreatmentvsHFDm, by = 'external_gene_name')

m <- df %>%
  tibble::column_to_rownames(var = 'external_gene_name_unique') %>%
  dplyr::select(FFA.control.1_S10:TEADsf.inh.3_S18) %>%
  scaleData(method = 'zscore') %>%
  dplyr::bind_cols(dplyr::select(df, DPNVsHFDm:PPTVsHFDm))

p <- Heatmap(m, width = unit(40, "mm"), height = unit(260, "mm"),
             row_split = factor(df$Pathway, levels = unique(df$Pathway)),
             column_split = c(rep(1,8), rep(2,4)),
             col = circlize::colorRamp2(breaks=seq(-2, 2, length.out=11),
                                        colors=colPals$RdBu),
             na_col = "grey80",
             row_title = unique(df$Pathway),
             row_title_gp = grid::gpar(fontsize = 6),
             row_labels = setNames(df$external_gene_name, df$external_gene_name_unique)[rownames(m)],
             row_names_gp = grid::gpar(fontsize = 6),
             column_names_gp = grid::gpar(fontsize = 6),
             cluster_row_slices = FALSE,
             cluster_column_slices = FALSE,
             border = T,
             gap = unit(1, 'mm'),
             row_dend_width = unit(5, 'mm'),
             column_dend_height = unit(5, 'mm'))

p <- Heatmap(m[1:8], width = unit(27, "mm"), height = unit(260, "mm"), name = 'zscore',
             row_split = factor(df$Pathway, levels = unique(df$Pathway)),
             col = circlize::colorRamp2(breaks=seq(-2, 2, length.out=11),
                                        colors=colPals$RdBu),
             row_title = unique(df$Pathway),
             row_title_gp = grid::gpar(fontsize = 6),
             column_names_gp = grid::gpar(fontsize = 6),
             cluster_row_slices = FALSE,
             cluster_column_slices = FALSE,
             border = T,
             gap = unit(1, 'mm'),
             row_dend_width = unit(5, 'mm'),
             column_dend_height = unit(5, 'mm')) +
  Heatmap(m[9:12], width = unit(13, "mm"), height = unit(260, "mm"), name = 'log2FC',
             col = circlize::colorRamp2(breaks=seq(-2, 2, length.out=11),
                                        colors=colPals$BrBG),
             na_col = "grey80",
             row_labels = setNames(df$external_gene_name, df$external_gene_name_unique)[rownames(m)],
             row_names_gp = grid::gpar(fontsize = 6),
             column_names_gp = grid::gpar(fontsize = 6),
             border = T,
             gap = unit(1, 'mm'),
             column_dend_height = unit(5, 'mm'))

draw(p)
```


Cholesterol metabolism
ANGPTL8
CYP7A1
LCAT
ABCB11
PCSK9
LDLR
LRP1
VDAC2
APOA4
AMPK signaling pathway
ACACB
FASN
ACACA
ADRA1A
FOXO3
PPARGC1A
SREBF1
G6PC1
PFKFB3
CCND1
SIRT1
HMGCR
IRS2
Alcoholic liver disease
TAB3
SREBF1
C3
C4A
ACACB
FASN
ACACA
FOXO3
PPARGC1A
CXCL2
CXCL8
CCND1
SIRT1
ALDH1B1
Glycerolipid metabolism
AKR1B10
AKR1B15
AGPAT2
GPAM
DGKD
PNPLA3
TKFC
ALDH1B1
Glucagon signaling pathway
CCND1
HBEGF
ABCB11
CYP2D6
NOTCH1
ADCY1
NOTCH2
ADCY9
GCGR
G6PC1
ACACA
EP300
SIK2
ACACB
CREBBP
PPARGC1A
GCK
SIRT1
ECM-receptor interaction
LAMC2
ITGB6
ITGA2
CD44
LAMB1
HSPG2
THBS1
TNXB
LAMA5
Bile secretion
CYP7A1
SLC22A7
HMGCR
ABCB11
SLC27A5
SLC9A1
LDLR
ADCY1
ADCY9
Insulin resistance
MLXIP
PPARGC1A
ACACB
MLXIPL
PPARGC1B
SREBF1
PPP1R3B
G6PC1
IRS2
SLC27A5
Bladder cancer
CXCL8
HBEGF
CCND1
MYC
THBS1
FGFR3
Cushing syndrome
AHR
CCND1
NR4A1
LDLR
ADCY1
CACNA1H
FZD4
PDE11A
KMT2A
CDK6
ADCY9
WNT11
PI3K-Akt signaling pathway
LAMC2
ITGB6
ITGA2
DDIT4
LAMB1
PDGFRA
MCL1
NR4A1
CCND1
IGF2
MYC
THBS1
GHR
EPHA2
FOXO3
TNXB
CDK6
LAMA5
G6PC1
FGFR3
FFA.control.3_S12
FFA.control.2_S11
FFA.control.1_S10
TEADsf.inh.1_S16
TEADsf.inh.3_S18
TEADsf.inh.2_S17
TEADap.inh.1_S13
TEADap.inh.2_S14
DIPVsHFDm
DPNVsHFDm
PPTVsHFDm
E2VsHFDm
zscore
2
1
0
-1
-2
log2FC
2
1
0
-1
-2

KEGG pathway genes changed by TEAD1i and ER activation

```
# entrez ID to ensembl ID matching
ann_genes <- getBM(
  mart=useDataset("hsapiens_gene_ensembl", useEnsembl(biomart = "ENSEMBL_MART_ENSEMBL", version = 109)),
  attributes=c("ensembl_gene_id", "entrezgene_id", "external_gene_name")) %>%
  filter(!is.na(entrezgene_id)) %>%
  filter(!external_gene_name == '')

# KEGG pathway genes
top12KEGG_all <- read.table(
  file = 'data/KEGG_pathways_april2023.txt',
  sep = '\t',
  header = TRUE,
  quote = '') %>%
  filter(KEGG_pathway_identifier %in% c("hsa04979", "hsa04152", "hsa04936", "hsa00561",
                                        "hsa01522", "hsa04922", "hsa04512", "hsa04976",
                                        "hsa04931", "hsa05219", "hsa04934", "hsa04151")) %>%
  filter(!duplicated(KEGG_ID)) %>%
  mutate(ensembl_gene_id = recode(KEGG_ID, !!!setNames(ann_genes$ensembl_gene_id,
                                                       ann_genes$entrezgene_id))) %>%
  mutate(GeneID_human = ensembl_gene_id) %>%
  inner_join(mouse_human_orthologs, by = 'GeneID_human') %>%
  filter(!(duplicated(GeneSymbol_human) | duplicated(GeneSymbol_human, fromLast = TRUE)))

top12_kegg <- readRDS('results/top12KEGGpathways_gene_content.rds')
top12_kegg_TEADap_inh_DEGs <- TEADap_inh_vs_control %>%
  filter(ensembl_gene_id %in% top12KEGG_all$ensembl_gene_id) %>%
  mutate(GeneID_human = ensembl_gene_id) %>%
  inner_join(mouse_human_orthologs, by = 'GeneID_human') %>%
  filter(!(duplicated(GeneSymbol_human) | duplicated(GeneSymbol_human, fromLast = TRUE)))

ER_activation_DEGs <- bind_rows(DEGs_mouse$filt[5:8]) %>%
  filter(!duplicated(ensembl_gene_id)) %>%
  mutate(GeneID_mouse = ensembl_gene_id) %>%
  inner_join(mouse_human_orthologs, by = 'GeneID_mouse') %>%
  filter(!(duplicated(GeneSymbol_mouse) | duplicated(GeneSymbol_mouse, fromLast = TRUE))) %>%
  filter(GeneID_human %in% top12KEGG_all$ensembl_gene_id)

top12KEGG_all_nonTEAD1i_DEGs <- top12KEGG_all %>%
  filter(!GeneSymbol_human %in% top12_kegg_TEADap_inh_DEGs$GeneSymbol_human)

# random_genes <- lapply(seq(1,1000), function(x) sample(x = top12KEGG_all_nonTEAD1i_DEGs$GeneSymbol_human, size = length(top12_kegg_TEADap_inh_DEGs
# saveRDS(random_genes, file = 'results/random_top12KEGG_nonTEADap_inh_sets.rds')
random_genes <- readRDS(file = 'results/random_top12KEGG_nonTEADap_inh_sets.rds')

# TEADap inhibitor DEG and non-DEG overlaps for genes in top 12 KEGG pathways that are are also
# changed by ER-agonist treatments in mouse HFD models
top12KEGG_nonTEADap_inh_DEG_ER_overlap <- lapply(random_genes, function(x){
  length(intersect(x, ER_activation_DEGs$GeneSymbol_human))/length(x)*100
}) %>% unlist() %>% as.data.frame() %>% dplyr::rename('perc_ER_activ' = '.')

top12KEGG_TEADap_inh_DEG_ER_overlap <- length(intersect(top12_kegg_TEADap_inh_DEGs$GeneSymbol_human, ER_activation_DEGs$GeneSymbol_human))/length(

print('Overlap for non-TEADap inhibitor DEG sets:')
```

```
## [1] "Overlap for non-TEADap inhibitor DEG sets:"
```

```
summary(top12KEGG_nonTEADap_inh_DEG_ER_overlap)
```

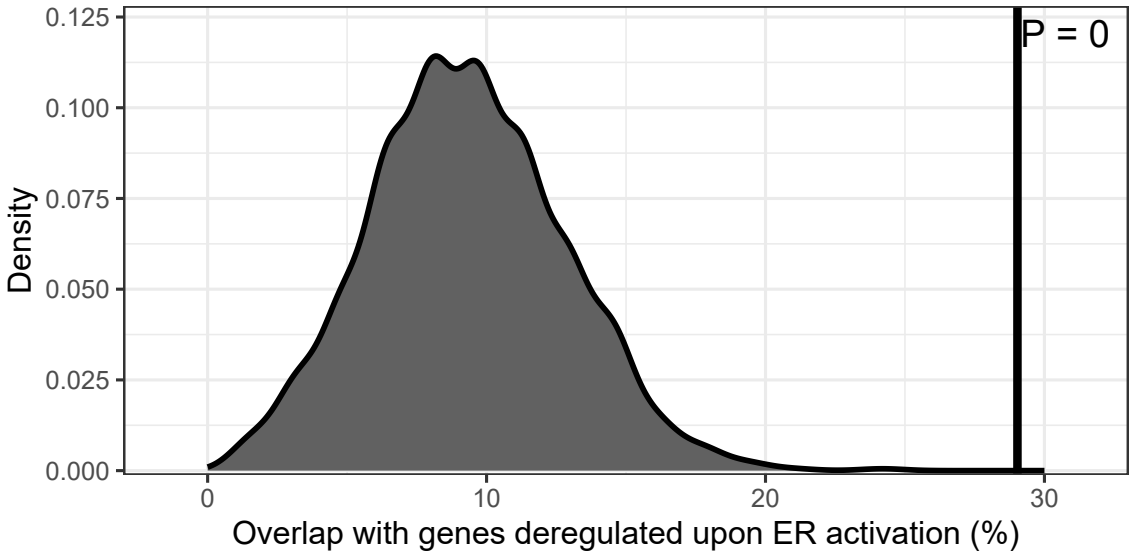
```
##  perc_ER_activ
##  Min.   : 1.613
##  1st Qu.: 6.452
##  Median : 9.677
##  Mean   : 9.305
##  3rd Qu.:11.290
##  Max.   :24.194
```

```
paste('Overlap for TEADap inhibitor DEG set:', top12KEGG_TEADap_inh_DEG_ER_overlap)
```

```
## [1] "Overlap for TEADap inhibitor DEG set: 29.0322580645161"
```

```
pval <- tailFraction(top12KEGG_TEADap_inh_DEG_ER_overlap,
                     top12KEGG_nonTEADap_inh_DEG_ER_overlap$perc_ER_activ,
                     tail = 'right')

ggplot(top12KEGG_nonTEADap_inh_DEG_ER_overlap, aes(x = perc_ER_activ)) +
  geom_density(lwd = 1, colour = 'black',
               fill = '#616161', alpha = 1) +
  geom_vline(xintercept = top12KEGG_TEADap_inh_DEG_ER_overlap, lwd = 1.5) +
  scale_x_continuous(limits = c(0,30),
```

```
                    breaks = c(0,10,20,30),
                    expand = expansion(mult = c(.1, .1))) +
  scale_y_continuous(expand = expansion(mult = c(.01, .12))) +
  annotate("text",  x=Inf, y = Inf, label = paste('P =',pval), vjust=1.5, hjust=1.2, size=5) +
  xlab('Overlap with genes deregulated upon ER activation (%)') +
  ylab('Density') +
  theme_bw(base_size = 12)
```



SessionInfo

```
sessionInfo()
```

```
## R version 4.2.1 (2022-06-23 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
##  [1] grid      tcltk     stats4    stats     graphics  grDevices utils
##  [8] datasets  methods   base
##
## other attached packages:
##  [1] ComplexHeatmap_2.14.0 GenomicRanges_1.50.1  GenomeInfoDb_1.34.9
##  [4] biomaRt_2.54.1        Mfuzz_2.58.0          DynDoc_1.76.0
##  [7] widgetTools_1.76.0    e1071_1.7-13          org.Hs.eg.db_3.16.0
## [10] AnnotationDbi_1.60.2  IRanges_2.32.0        S4Vectors_0.36.0
## [13] Biobase_2.58.0        BiocGenerics_0.44.0   lubridate_1.9.2
## [16] forcats_1.0.0         stringr_1.5.0         dplyr_1.1.0
## [19] purrr_1.0.1           readr_2.1.4           tidyr_1.3.0
## [22] tibble_3.2.1          ggplot2_3.4.2         tidyverse_2.0.0
## [25] clusterProfiler_4.6.2
##
## loaded via a namespace (and not attached):
##   [1] circlize_0.4.15        shadowtext_0.1.2       fastmatch_1.1-3
##   [4] BiocFileCache_2.6.1    plyr_1.8.8             igraph_1.3.5
##   [7] lazyeval_0.2.2         splines_4.2.1          BiocParallel_1.32.3
##  [10] digest_0.6.30          foreach_1.5.2          yulab.utils_0.0.6
##  [13] htmltools_0.5.5        GOSemSim_2.24.0        viridis_0.6.3
##  [16] GO.db_3.16.0           fansi_1.0.3            magrittr_2.0.3
##  [19] memoise_2.0.1          cluster_2.1.4          doParallel_1.0.17
##  [22] tzdb_0.4.0             Biostrings_2.66.0      graphlayouts_0.8.4
```

```
##  [25] matrixStats_0.63.0     timechange_0.2.0       enrichplot_1.18.4
##  [28] prettyunits_1.1.1      colorspace_2.0-3       blob_1.2.4
##  [31] rappdirs_0.3.3         ggrepel_0.9.2          xfun_0.39
##  [34] crayon_1.5.2           RCurl_1.98-1.9         jsonlite_1.7.2
##  [37] scatterpie_0.2.1       iterators_1.0.14       ape_5.6-2
##  [40] glue_1.6.2             polyclip_1.10-4        gtable_0.3.3
##  [43] zlibbioc_1.44.0        XVector_0.38.0         GetoptLong_1.0.5
##  [46] shape_1.4.6            scales_1.2.1           DOSE_3.24.2
##  [49] DBI_1.1.3              Rcpp_1.0.9             viridisLite_0.4.2
##  [52] progress_1.2.2         clue_0.3-63            gridGraphics_0.5-1
##  [55] tidytree_0.4.4         bit_4.0.5              proxy_0.4-27
##  [58] httr_1.4.6             fgsea_1.24.0           RColorBrewer_1.1-3
##  [61] pkgconfig_2.0.3        XML_3.99-0.12          farver_2.1.1
##  [64] dbplyr_2.3.3           utf8_1.2.2             labeling_0.4.2
##  [67] ggplotify_0.1.1        tidyselect_1.2.0       rlang_1.1.1
##  [70] tkWidgets_1.76.0       reshape2_1.4.4         munsell_0.5.0
##  [73] tools_4.2.1            cachem_1.0.6           downloader_0.4
##  [76] cli_3.4.1              generics_0.1.3         RSQLite_2.2.19
##  [79] gson_0.1.0             evaluate_0.21          fastmap_1.1.0
##  [82] yaml_2.3.7             ggtree_3.6.2           knitr_1.43
##  [85] bit64_4.0.5            tidygraph_1.2.2        KEGGREST_1.38.0
##  [88] ggraph_2.1.0           nlme_3.1-162           aplot_0.1.10
##  [91] xml2_1.3.5             compiler_4.2.1         rstudioapi_0.15.0
##  [94] filelock_1.0.2         curl_5.0.1             png_0.1-8
##  [97] treeio_1.22.0          tweenr_2.0.2           stringi_1.7.8
## [100] highr_0.10             lattice_0.20-41        Matrix_1.5-3
## [103] vctrs_0.6.2            pillar_1.9.0           lifecycle_1.0.3
## [106] GlobalOptions_0.1.2    data.table_1.14.6      cowplot_1.1.1
## [109] bitops_1.0-7           patchwork_1.1.2        qvalue_2.30.0
## [112] R6_2.5.1               gridExtra_2.3          codetools_0.2-19
## [115] MASS_7.3-60            rjson_0.2.21           withr_2.5.0
## [118] GenomeInfoDbData_1.2.9 parallel_4.2.1         hms_1.1.3
## [121] ggfun_0.1.1            HDO.db_0.99.1          class_7.3-22
## [124] rmarkdown_2.23         Cairo_1.6-0            ggforce_0.4.1
```